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A 72 kD trophoblast glycoprotein defined by a monoclonal antibody

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Summary A novel trophoblast cell surface antigen has been defined by a monoclonal antibody 5T4, raised following immunisation with wheat germ agglutinin (WGA) purified glycoproteins from deoxycholate (DOC) solubilised human syncytiotrophoblast plasma membrane (StMPM). The distribution of the antigen was determined by indirect immunoperoxidase staining of sections of normal organ and placental tissues as well as immunofluorescence and radiobinding assays with a wide variety of cell lines representing differing normal and tumour cell types. In frozen sections of normal full term placenta, 5T4 is strongly expressed only by the syncytiotrophoblast, some extravillous cytotrophoblast and the amniotic epithelium. The 5T4 antigen is apparently not expressed by any maternal component of the placenta nor is it detected in adult liver, lung, bronchus, heart, testis, ovary, brain, or muscle. The antigen is apparently expressed by several specialised epithelia. Immunoprecipitation of radiolabelled StMPM indicated that 5T4 molecules are glycoproteins of mol. wt of ~72 kD on SDS-PAGE. 5T4 antigen is selectively expressed by diverse tumour cell lines, including those of developmental origin. The molecular characteristics, relatively restricted normal tissue distribution and expression by certain tumour cell types make this antigen worthy of future study for use as a diagnostic marker of malignancy.

Trophoblast demonstrates some functional properties of neoplastic tissue, *viz.* invasiveness of host tissue and escape from immunological surveillance. Several monoclonal antibodies to trophoblast membrane proteins have been described. In terms of cancer research, the rationale behind this approach has been to identify 'oncofoetal' antigens present on both trophoblast and neoplastic cells (Johnson, 1984). If such antigens were restricted to neoplastic tissues, then these reagents would be potentially useful in diagnosis, tumour localisation and drug targeting. Of those monoclonal antibodies that do identify trophoblast oncofoetal antigens, relatively few have been fully characterised. A variety of monoclonal antibodies have been shown to be reactive with the placental alkaline phosphatase (PLAP), and these have shown the greatest clinical potential (McLaughlin, 1986). The low level of PLAP in normal non-pregnant sera, and restricted tissue distribution has been useful in monitoring some ovarian carcinomas by a serum assay (McDicken *et al.*, 1985) and radio-imaging (Epenetos *et al.*, 1985; Critchley *et al.*, 1986). However, PLAP-reactive monoclonal antibodies are not reactive with all ovarian carcinomas. Clearly there is a place for further reagents against different molecular species which show a different and/or wider tumour cell type reactivity. Here we describe a novel trophoblast antigen which is also expressed by some tumour cell lines.

Materials and methods

Purification of syncytiotrophoblast glycoproteins

StMPM was purified from full term human placentae, obtained within 1 h post partum, by the method of Smith *et al.* (1974). The StMPM pellet was solubilised in 0.5% DOC in tris-buffered saline (TBS, 0.15 M NaCl, 25 mM tris, pH 8.0) containing 0.1 mM phenylsulphonylmethyl fluoride (PMSF) and centrifuged at 14,000 *g* for 10 min. The WGA-reactive glycoproteins were then purified by incubation of the supernatant with WGA-Sepharose (5 mg ligand ml^{-1} Sepharose) for 1 h at room temperature. The beads were washed extensively in TBS/0.5% DOC, and the specifically bound glycoproteins eluted in 5 ml of 0.2 M N-acetyl glucosamine (Sigma) in TBS. The eluted fraction was extensively dialysed against 30 mM ammonium bicarbonate (pH 7.9), and lyophilised.

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Generation of monoclonal antibody

A male BALB/c mouse was immunised by 6 i.p. injections of WGA-purified StMPM glycoproteins (100–200 μg/injection). Spleen cells were fused with NS1 murine myeloma cells (Kohler & Milstein, 1975), and the cells plated out in 24 well Linbro plates at 7×10^5 cells/well. After 2 weeks, wells were assayed for StMPM reactive antibody by immunodotting. Positive clones were picked directly and further subcloned by limiting dilution. The antibody subclass was determined by double radial diffusion using a monoclonal isotype typing kit (Serotec, Bicester, UK).

Cell culture

Details of the cell lines are given in Table III. Standard tissue culture media, alpha Dulbecco's modified Eagle's medium (DMEM), DMEM or RPMI supplemented with antibiotics and 10–20% foetal calf serum (Gibco) were used.

Radioactive labelling of membranes and cells

Near confluent cell cultures of AV-3 cells were radiolabelled for 15–18 h with ^{3}H-glucosamine (20 μCi ml^{-1}) (Amersham International) in RPMI containing 10% dialysed FCS. Metabolically labelled cells were collected and immunoprecipitated as follows: cells were removed from tissue culture flasks by incubation in 0.1 M EGTA-PBS, washed in PBS (Dulbecco's-A) and then solubilised for 30 min at 4°C in 0.5% (v/v) NP40 in tris-buffered saline (TBS, 0.15 M NaCl, 25 mM Tris, pH 8.0) containing 0.1 mM PMSF. Non-solubilised cellular components were removed by centrifugation at 14,000 *g* and the amount of radioactivity incorporated into protein was determined following precipitation with 10% trichloroacetic acid.

Cell-surface labelling by the lactoperoxidase-^{125}I method together with the techniques of immunoprecipitation and SDS-PAGE were carried out as previously described; high mol. wt standards (Sigma), red blood cell membrane proteins or ^{14}C-methylated protein mixtures (Amersham International) were used as marker proteins (Thompson *et al.*, 1984; Stern *et al.*, 1984, 1986). Tritiated sodium borohydride labelling of cell surface glycoproteins was carried out as described by Axelsson *et al.* (1978). Autoradiography and fluorography were as described in Thompson *et al.* (1984) using pre-flashed Fuji X-ray film.

Immunoperoxidase and immunofluorescence labelling

Immunoperoxidase staining of frozen tissue sections was carried out by the method of Bulmer & Sunderland (1983). Tissues were obtained as soon as possible post-mortem, always within 12 h, and processed immediately. Indirect immunofluoresecence with cell suspensions was as described previously (Thompson *et al.*, 1984). A monoclonal antibody generated in this laboratory against a widely expressed human antigen (mAb 1D2) was used as positive control.

Radiobinding assay of cell surface antigen expression

Cells were harvested with either EGTA-PBS or EGTA/trypsin, washed and resuspended in Earle's buffered saline solution (EBSS) with 0.5% bovine serum albumin and 0.1% sodium azide at 2×10^6 cells ml^{-1}. The suspensions were plated out at 50 μl (10^5 cells)/well in microtitre plates. Fifty μl mAb/well were added and incubated at room temperature for 1 h. The cells were washed and 5×10^5 CPM of ^{125}I-labelled $(Fab')_2$ fragments of sheep anti-murine immunoglobulin (Amersham International) added. Following incubation for 1 h at room temperature, the cells were washed, harvested, and bound radioactivity determined on a gamma-counter. Assays were carried out in quadruplicate. Results are expressed as a ratio of specifically bound radioactive cpm relative to CPM with negative control antibodies. In some experiments 10^7 cells were incubated with 1 ml of fixative (buffered 10% formalin, Bouins' fixative, 0.25% gluteraldehyde, absolute ethanol or PBS control) for 30 min at room temperature and washed in EBSS. After incubation in 0.5% BSA in EBSS for 30 min, the cells were then processed as described above.

Preparation of crude membrane from normal human tissues

Tissues were obtained within 12 h of death, and processed immediately. Tissue (10–20 g) was finely chopped, rinsed, and homogenised in 10–20 ml of ice-cold PBS containing 5 mM $MgCl_2$ and 0.1 mM PMSF with 20 strokes of a Dounce homogeniser. The homogenate was centrifuged at 10,000 *g* for 20 min, the pellet discarded and the supernatant centrifuged at 100,000 *g* for 1 h. This pellet was solubilised in 0.5% (w/v) DOC/TBS containing 0.1 mM PMSF and unsolubilised material pelleted by centrifugation at 14,000 *g*. The protein concentration of the supernatant was determined by the method of Lowry *et al.* (1951). Membranes from 12 h old placentae were prepared identically and acted as positive controls.

Gel filtration

StMPM protein (50 mg) was solubilised in 6.5 ml of 1.0% (w/v) DOC/TBS containing 0.1 mM PMSF, centrifuged at 100,000 *g* for 30 min, and the supernatant fractionated over S200 Sephacryl (Pharmacia). Column size was 90×2 cm, running buffer was 0.1% (w/v) NaDOC/TBS containing 0.1 mM PMSF. Flow rate was 17 ml h^{-1}. Fraction size was 3.3 ml. The column was calibrated with the following proteins; Equine ferritin (Sigma), IgG (Kabi), transferrin (Sigma), Bovine serum albumin (Sigma) and ovalbumin (Sigma). Fractions were assayed for 5T4 antigen in ELISA and immunodot.

ELISA and immunodot

ELISA plates (Dynatech) were activated by 1 h incubation with 100 μl/well of PBS containing 0.25% gluteraldehyde (BDH), the plates washed with PBS, and 100 μl/well of undiluted or 10-fold diluted fractions from gel filtration bound to the plates by overnight incubation at 4°C. Following washing, the plates were incubated with 1% BSA/TBS as blocking agent. ELISA was then carried out as described (Johnson *et al.*, 1981). Immunodotting on nitrocellulose was carried out using the Bio-Rad Dot-Blot apparatus. Fractions from gel filtration were loaded at 10 μl and 100 μl/dot. NaDOC solubilised plasma membrane was loaded in the range of 50 μg–12.5 ng protein/dot. The following antigens were loaded at 1 μg protein/dot; transferrin (Sigma), PLAP (gift of Dr P.J. McLaughlin), human placental lactogen (HPL) (Sigma), calmodulin (Sigma), IgG (Miles Ltd.), albumin (Miles Ltd.) and normal human sera. The nitrocellulose sheet was blocked with 3% (w/v) BSA (Sigma) in TBS and processed as described previously (Webb *et al.*, 1985). In both ELISA and immunodot, mAb 1D2 was used as positive control.

Enzymatic digestion

StMPM membranes (~1 mg protein) were treated overnight at 37°C with either 2 mg trypsin (Boehringer), 2 mg pronase (Boehringer), 0.1 U neuraminidase (Behringwerke) in 300 μl PBS or 10 U ml^{-1} N-glycanase (Genzyme) in buffer containing final concentrations as follows: 0.17% SDS; 0.2 M tris-HCl, pH 8.7; 10 mM 1,10-phenanthroline hydrate (in methanol); 1.25% NP-40 (Plummer *et al.*, 1984). The treated membranes were solubilised in DOC/TBS and 5T4 residual antigenicity assayed by dot-blot. 5T4 immunoprecipitates of detergent solubilised ^{125}I-radiolabelled StMPM were eluted from protein-A-Sepharose with 0.5% SDS in water and incubated overnight at 37°C with or without 10 U ml^{-1} N-glycanase in buffer as above. Digests were subjected to reduced SDS-PAGE and autoradiography.

Results

The monoclonal antibody 5T4 is a murine IgG1. All work detailed in this study was carried out using subclone 5T4.B8. The preliminary screen by immunodot showed that the antigen recognised was none of the following major proteins associated with the trophoblast; IgG, transferrin, PLAP, HPL, albumin, calmodulin, nor was it detectable in serum.

Tissue distribution

5T4 antigen expression in first trimester and full term placentae was investigated using indirect immunoperoxidase staining of frozen sections. Figure 1 illustrates antigen expression in term villous placenta as assessed by immunohistology of frozen sections. Villous trophoblast was strongly labelled by mAb 5T4, whereas the stroma was negative. There was specific labelling of the amniotic epithelium and extravillous cytotrophoblast of the chorion laeve but not of the amniotic mesenchyme or maternal decidua (Figure 1c, d). Appropriate positive and negative controls are also shown; mAb 1D2 labelled all parts of villi (Figure 1a), mAb H316 labelled trophoblast but was not specific for this tissue type (Figure 1b; Stern *et al.*, 1986); negative controls were unlabelled (Figure 1e, f). Extravillous cytotrophoblast in the placental bed was also labelled by mAb 5T4; no other element of the term placenta was 5T4 antigen-positive. Similar analysis of first trimester villous tissue revealed antigen expression by both syncytiotrophoblast and cytotrophoblast (data not shown). The earliest stage examined for 5T4 expression was in a chorionic villous biopsy at 9 weeks gestation which was positive by indirect immunofluorescence (with Dr Bruce Smith, Jefferson, Philadelphia). This level of analysis suggests that 5T4 antigenic molecules are expressed by representatives of all subpopulations of trophoblastic cells.

5T4 was unreactive with the following non-pregnant tissues examined in immunohistology; spleen, heart, brain, liver, lung, bronchus, skeletal muscle, testis or ovary. Glomeruli in the kidney, villi of the small intestine, bladder epithelium, basal layer of the epidermis, endometrial glands

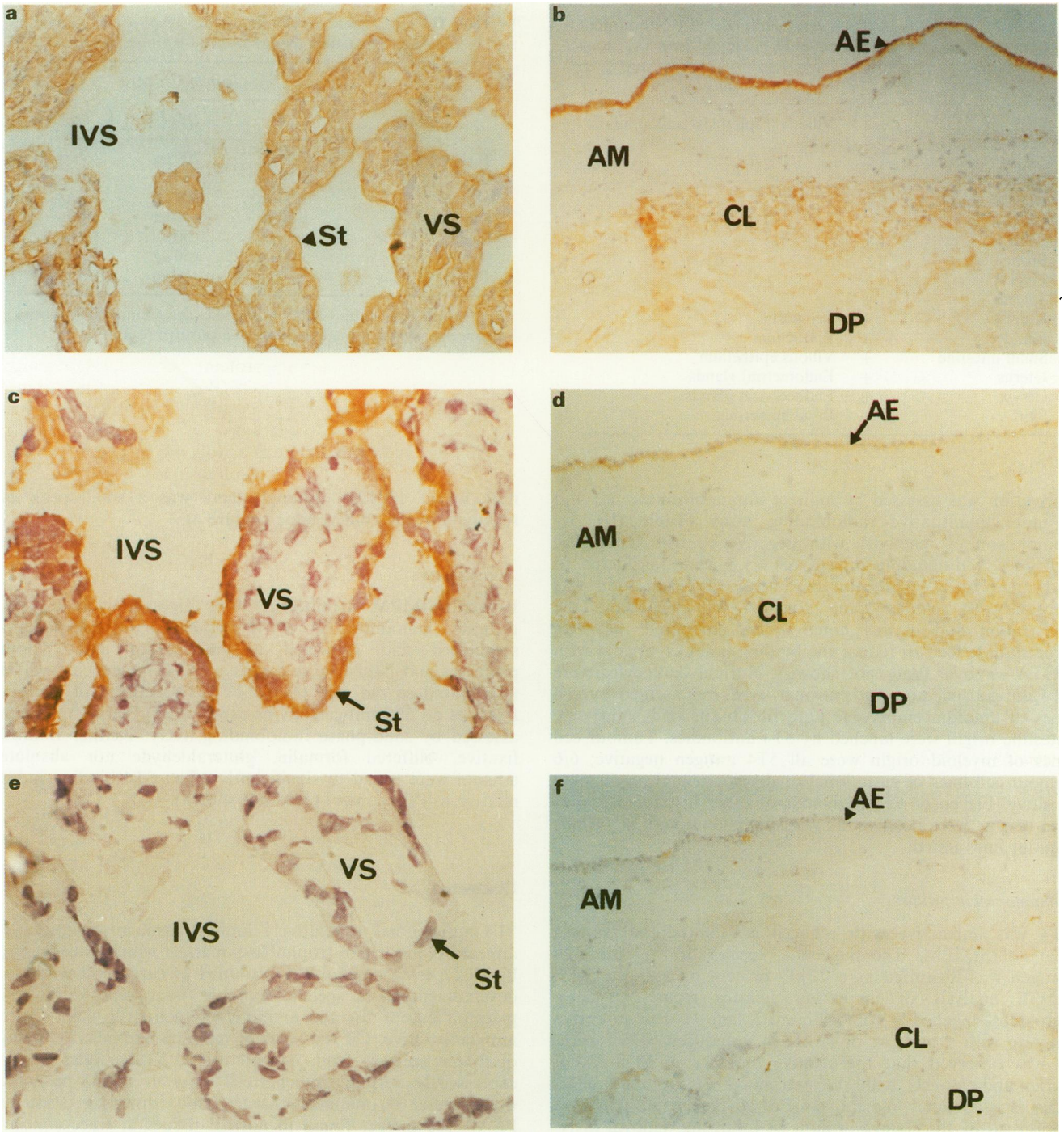

Figure 1 Expression of 5T4 antigen in placenta. Immunohistology of term chorionic villi (a, c, e) or amnio-chorion (b, d, f) with normal mouse serum (e, f) or monoclonal antibodies 1D2 (a), H316 (b) or 5T4 (c, d) followed by rabbit anti-murine immunoglobulin peroxidase conjugate. Sections were counterstained with haemalum. IVS, intervillous space; St, syncytiotrophoblast; VS, villous stroma; AE, amniotic epithelium; AM, amniotic mesenchyme; CL, chorion laeve; DP, decidua parietalis. 5T4 shows specific labelling of villous trophoblast and extravillous cytotrophoblast of the chorion laeve as well as amniotic epithelium. Positive control mAb 1D2 labels all cell types; mAb H316 labels trophoblast of the chorion laeve and amniotic epithelium. Normal mouse serum shows no labelling.

of non-pregnant uterus and endocervical glands showed some specific labelling with mAb 5T4. Some small vessels in various tissues appeared to be weakly stained. Table I summarises 5T4 reactivity assayed by immunohistology of frozen tissue sections.

To further examine 5T4 expression, a semi-quantitative assay of 5T4 antigen on isolated membranes of some of the above tissues was assessed using solubilised proteins in an immunodot assay. 5T4 was still reactive with full term placental plasma membrane protein at an antigen concentration of 50 ng/dot. In contrast to the widely distributed antigen recognised by mAb 1D2, 5T4 was not specifically reactive with any other tissue tested (ovary, testis, kidney, brain, liver and muscle) at all antigen concentrations used (up to 50 μg/dot). From this it was concluded that these normal non-gestational tissues express 5T4 antigen at $\sim$1,000-fold lower concentration than full-term placenta on a weight of crude membrane protein basis. This relative level of expression is comparable with PLAP as measured using mAb H317 (Table II).

Expression by cell lines

5T4 antigen expression by cell lines of normal and neoplastic

Table I Reactivity of monoclonal antibody 5T4 with normal human tissues assessed by immunohistology of frozen sections

Tissue	*Result*	
Placenta	+ + +	Villous trophoblast and amnion
Brain	−	
Ovary	−	
Testis	−	
Skeletal muscle	−	
Heart	−	
Lung	−	
Bronchus	−	
Liver	−	
Spleen	−	
Kidney	+	Glomeruli
Bladder	+	Epithelium
Small intestine	+	Villous epithelium
Uterus	+	Endometrial glands
Cervix	+	Endocervical glands
Skin	+	Basal epidermis

Table II Expression of 5T4 and other trophoblast antigens by non-pregnancy tissues assessed by immunodot

	Immunodot titre		
Tissue	*5T4*	*H317*	*1D2*
Term placenta	50 ng	200 ng	50 ng
Brain	> 50 μg	> 50 μg	200 ng
Muscle	> 50 μg	> 50 μg	200 ng
Kidney	> 50 μg	> 50 μg	100 ng
Liver	> 50 μg	> 50 μg	100 ng
Ovary	> 50 μg	> 50 μg	100 ng
Testis	> 50 μg	> 50 μg	100 ng

Results of immunodot expressed as minimum antigen concentration required to produce a positive result.

derivation was assessed by indirect immunofluorescence and a more quantitative radiobinding assay (Table III). By comparison of reactivity with negative control xenogeneic cell lines, radiobinding indices of >1.5 were considered to indicate positive expression of antigen. Trypsinisation was necessary to remove some attached cell lines from the substratum and it was noted where compared that this procedure tended to reduce the binding index compared with EGTA removal (data not shown). Normal leukocytes were 5T4 antigen negative and 'normal' types represented by cell lines of amnion, embryonic lung fibroblasts and embryonic intestine origin were labelled by 5T4 antibodies. Tumour cell lines of myeloid origin were all 5T4 antigen negative; 6/6 tumour cell lines of gestational or developmental origin were positive. Eleven of 15 carcinomas of other histological types and origins were positive, as was one glioma and 1/3 Wilms tumour lines tested.

Immunoprecipitation

5T4 was unreactive with reduced and unreduced western blots of StMPM. The molecular species bearing the 5T4 antigen was identified as a 72 kD protein by reduced SDS-PAGE analysis of immunoprecipitates from ^{125}I-lactoperoxidase-labelled StMPM (Figure 2, lane 1). The molecules migrate with a mol. wt of 69 kD in unreduced SDS-PAGE. It was observed that the relative mobility in SDS-PAGE varies anomalously with the percentage of the acrylamide. This is sometimes indicative of a glycoprotein, which is confirmed by the change in mol. wt following removal of N-linked sugars by digestion with N-glycanase, yielding a molecule of 42 kD (Figure 2, lane 2).

5T4 glycoprotein can be labelled by reduction with tritiated sodium borohydride either after periodate oxidation of sugar residues or galactose oxidase/neuraminidase treatment. These latter treatments change the relative mobility in SDS-PAGE as compared with ^{125}I-labelled 5T4 antigen (Figure 3). AV-3, Tera-2, MRC-5, Hep-2, HN5, HT29 cell lines all express a molecule of similar mol. wt to that on StMPM as judged by SDS-PAGE of immunoprecipitates of surface iodinated cells; the antigen has been immunoprecipitated from AV-3 cells metabolically labelled with tritiated glucosamine (data not shown).

Gel filtration

In order to investigate any association of 5T4 antigen with itself or any other protein, DOC solubilised StMPM was subjected to gel filtration over S200 Sephacryl run in the presence of detergent, and the fractions assayed for 5T4 reactivity in ELISA. 5T4 antigen eluted with an apparent mol. wt of 120 kD, although there was a small peak of reactivity in the void volume (Figure 4).

Antigenicity

Isolated StMPM membranes were digested with trypsin, pronase, neuraminidase or N-glycanase, the components solubilised and subjected to immunodot assay. Both proteases and N-glycanase destroyed 5T4 antigenicity, whilst neuraminidase did not (Table IV). The effects of various fixatives on 5T4 antigenicity as expressed by Tera-2 cells was assessed by solid-phase radiobinding assay. Neither Bouins' fixative, buffered formalin, gluteraldehyde nor absolute ethanol were found to significantly affect 5T4 binding index relative to PBS control (data not shown).

Discussion

5T4 antigen has a relatively limited tissue distribution. It appears to be a pan-trophoblast marker which is expressed by all types of trophoblast examined as early as 9 weeks of development. It is specific for this tissue type within the placenta except for the amniotic epithelium which is also antigen positive. On the basis of immunoperoxidase staining of frozen sections from normal tissue, 5T4 antigen is also expressed by certain epithelial cell types. It should be noted that several 'trophoblast-characteristic' antigens, such as PLAP, are in fact found in normal tissues at trace concentrations (McLaughlin, 1986). Using a solid phase immunoassay to quantitate the expression of 5T4 relative to normal tissue, 5T4 antigen was found in placental plasma membrane in at least a 1,000-fold higher concentration than that found in other normal tissues tested. However, this level of sensitivity would not necessarily detect expression in minor subpopulations of cells within a given tissue.

Several antibodies have exhibited a similar pattern of reactivity with normal epithelial tissues, for example HMFG1 and 2 (Taylor-Papadimitriou *et al.*, 1981; Wilkinson *et al.*, 1984), and CA 1, 2 and 3 (Bramwell *et al.*, 1985), but this has not limited their use in immunoscintigraphy (Pateisky *et al.*, 1985) or diagnosis of neoplasia (Warr & Cruickshank, 1987). In this respect, 5T4 is reactive with tumour cell lines of a diverse, but select origin, including those of a developmental nature, such as choriocarcinoma and embryonal carcinoma. The reason for 5T4 antigen expression by cell lines of such apparent diversity of tissue is not clear; the normal cell line types tested are all of embryonic origin. The lack of reactivity with tumour cell lines derived from lung, bronchus and lymphoid tissue is

Table III Reactivity of mAb 5T4 with normal cells and transformed cell lines in cell-surface immunofluorescence and radiobinding assay

Cell	Origin	Type	Result: Fluorescence	Result: Binding index	Reference
AV-3	Amnion	Epithelial	+	3.1	McLaughlin *et al.*, 1982
WISH	Amnion	Epithelial	nt	(3.4)	Gift of P. McLaughlin, Liverpool
MRC-5	Fibroblasts	Embryonic	+	3.8 t	Jacobs *et al.*, 1970
Flow 7000	Fibroblasts	Embryonic	nt	(2.9)	Gift of P. McLaughlin, Liverpool
I407	Intestine	Embryonic	+	nt	Gift of A. Smith, Clatterbridge
PBL	Peripheral blood	Leucocytes	−	nt	(1)
UC729/6	B-cell	Myeloma	−	nt	Gift of A. Smith, Liverpool
HMI	B-cell	Myeloma	−	nt	Gift of A. Smith, Liverpool
RAJI	B-cell	Lymphoblastoid	−	1.2	Pulvertaft, 1964
BSM	B-cell	Lymphoblastoid	−	1.2	Gift of Dr C. Graham, Oxford
Daudi	B-cell	Burkitt's lymphoma	−	1.2	Klein *et al.*, 1967
B27	B-cell	EBV-lymphoblastoid	−	1.1	Gift of Prof. C, Hart, Liverpool
Molt-4	T-cell	Leukaemia	−	nt	Minowada *et al.*, 1972
K562	T-cell	Erythroleukaemia	−	1.2	Andersson *et al.*, 1979
GCCM/15	Brain	Glioma	+	5.2 t	Gift of Dr T. Alderson, London
Hep-2	Larynx	Carcinoma	+	(5.0)	Moore *et al.*, 1955
HN2	Larynx	Carcinoma	+	(1.5) t	Easty *et al.*, 1981
HN4	Larynx	Carcinoma	+	3.0 t	Easty *et al.*, 1981
HN1	Tongue	Carcinoma	+	2.9 t	Easty *et al.*, 1981
HN5	Tongue	Carcinoma	+	3.1 t	Easty *et al.*, 1981
IPT	Bronchus	Carcinoma	−	1.2 t	Kumar *et al.*, 1983
IPTV2	Bronchus	Carcinoma	−	1.3 t	Walker *et al.*, 1984
N417	Small lung	Carcinoma	−	1.2	Gift of Dr T. Alderson, London
6CT	Cervix	Carcinoma	+	2.2	Daniels *et al.*, 1984
E1Co	Breast	Carcinoma	nt	1.7	Gift of P. McLaughlin, Liverpool
EJ	Bladder	Carcinoma	+	nt	O'Toole, *et al.*, 1983
A431	Vulva	Carcinoma	+	4.2 t	Fabricant *et al.*, 1977
HT29	Colon	Carcinoma	+	3.4 t	Gift of A. Smith, Liverpool
Mawi	Colon	Carcinoma	−	nt	Gift of A. Smith, Liverpool
Chang	Liver	Carcinoma	nt	(4.1)	Gift of P. McLaughlin, Liverpool
Tera-1	Testis	Teratocarcinoma	+	(2.6)	Fogh and Trempe, 1975
Tera-2	Testis	Teratocarcinoma	+	4.2	Thompson *et al.*, 1984
2102Ep	Testis	Teratocarcinoma	+	(3.5)	Andrews *et al.*, 1984
PA-1	Ovary	Teratocarcinoma	+	(4.1)	Zeuthen *et al.*, 1980
BeWo	Chorion	Choriocarcinoma	+	(5.2)	Patillo and Gey, 1968
JAr	Chorion	Choriocarcinoma	+	(4.9)	Patillo *et al.*, 1971
SK-NEP	Kidney	Wilm's tumour	−	(1.2)	Fogh and Trempe, 1975
Gos.1.8.1	Kidney	Wilm's tumour	−	(1.4)	Gift of Dr C. Graham, Oxford
GM3808	Kidney	Wilm's tumour	+	(5.1) t	Gift of Dr T. Alderson, London

Cells harvested with EGTA alone or EGTA-trypsin (t). Cells incubated with mAb 5T4 followed by fluorescein-conjugated sheep anti-murine Ig (immunofluorescence) or ^{125}I rabbit anti-mouse immunoglobulin (Binding assay). Results expressed as positive immunofluorescence or binding index relative to negative control. Standard deviation of 4 replicates was $<10\%$; variation between 2–4 experiments was generally $<10\%$. Figures in parentheses represent results from a single experiment. (1) PBL isolated from peripheral blood by centrifugation over Ficoll-hypaque. nt = not tested.

consistent with the immunohistology of the normal tissue types. Other antigen positive tumour cell lines may have been derived from an epithelial component of normal tissue or represent re-expression of embryonic antigen on tumour cells. Several trophoblast antigens have been reported to exhibit a pattern of expression by tumour cell types apparently not detected in the normal cell counterpart (McLaughlin *et al.*, 1982). In the study by Rettig *et al.* (1985), a series of six monoclonal antibodies were generated against choriocarcinoma cells, one of which was reactive with neoplastic, but not normal, kidney cells; the other mAbs did not demonstrate such a selective expression.

Several trophoblast associated antigens have been reported in the literature to be expressed on tumour cell lines. 5T4 antigen does, however, appear to be novel. On the basis of reactivity in dot-blots and other criteria, we have specifically excluded PLAP and transferrin as the 5T4 antigen. On the basis of mol. wt in reduced gels, we have further excluded transferrin receptor (Trowbridge *et al.*, 1984), insulin receptor (Ullrich *et al.*, 1985), EGF receptor (Waterfield *et al.*, 1982), HMFG1 and 2 (Burchell *et al.*, 1983), CA (Wiseman *et al.*, 1984), CEA (Krantz *et al.*, 1979), alpha foeto-protein (Ruoslahti, 1979) and all of the placental specific proteins reviewed by Bohn *et al.* (1983). On the basis of mol. wt and cell line reactivity, none of the monoclonal antibodies described by Lipinski *et al.* (1981), Sunderland *et al.* (1981), McLaughlin *et al.* (1982), Loke *et al.* (1984), Travers and Bodmer (1984), Rettig *et al.* (1985), Yamashita *et al.* (1986) or Mueller *et al.* (1986) appear to recognise this antigen.

The 5T4 antigen is carried by glycoprotein molecules of 72 kD on syncytiotrophoblast microvillous plasma membranes but appears on molecules of similar mol. wt from several different cell lines including some choriocarcinomas. The molecules are sialylated and have approximately 30 kD of the apparent mol. wt due to N-linked carbohydrate structures as judged from removal of the latter by N-glycanase endoglycosidase.

5T4 appears to exist on the cell surface as a monomeric protein. Firstly, 5T4 antigen elutes with an apparent mol. wt in gel filtration of 120 kD, an increase consistent with the addition of a detergent shell, and inferring that 5T4 is not associated non-covalently with any other large molecules. Additionally, reduction with 2-mercaptoethanol does not substantially alter the apparent mol. wt of the 5T4 radio immunoprecipitate, as would be the case if it were disulphide bonded to another protein.

The pattern of expression of 5T4 is similar to that of the

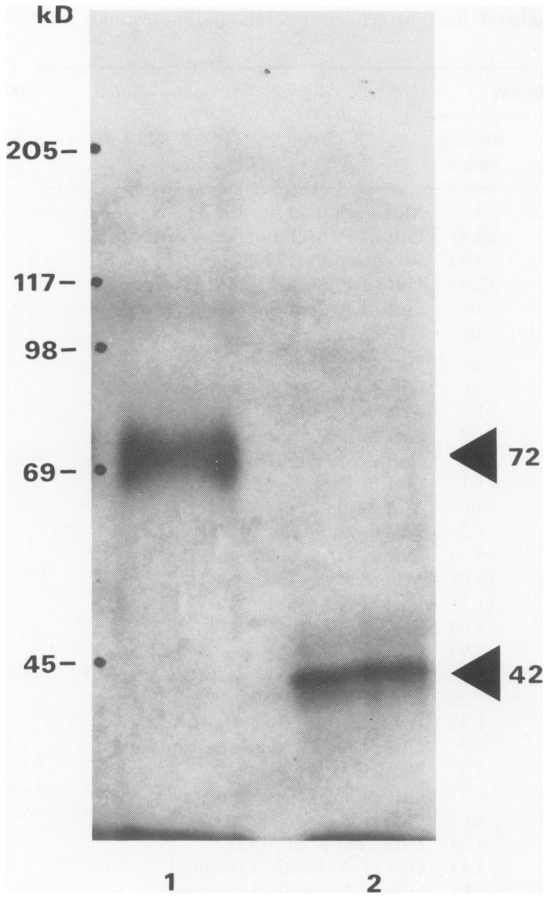

Figure 2 Immunoprecipitation of 5T4 molecules from StMPM. Autoradiography of SDS-PAGE analysis of 5T4 immunoprecipitates of NP-40 solubilised ^{125}I-lactoperoxidase labelled StMPM (lane 1) and following digestion with N-glycanase (lane 2). 8% gel.

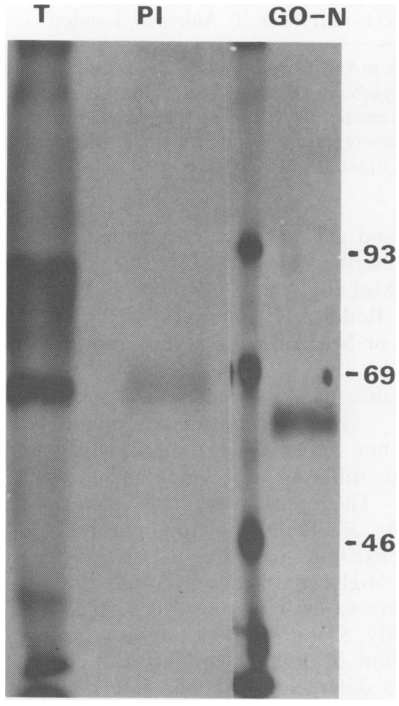

Figure 3 Fluorography of reduced SDS-PAGE of 5T4 immunoprecipitates from StMPM labelled with NaB^3H_4 following treatment with either periodate (PI) or galactose oxidase and neuraminidase (GO-N). 10% gel. T is total radiolabelled glycoprotein following periodate treatment.

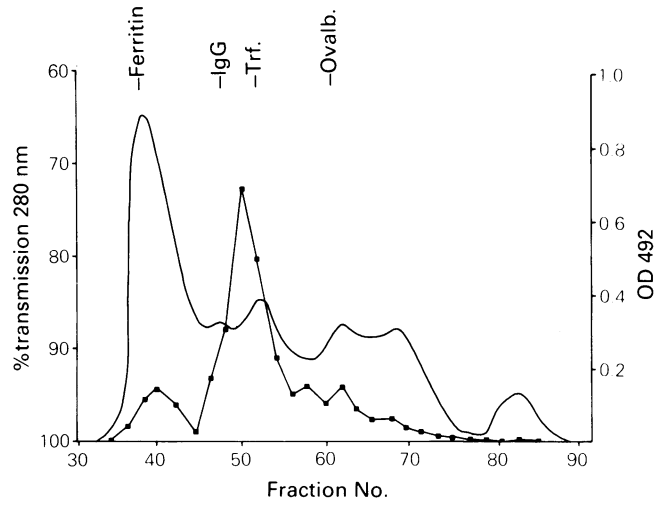

Figure 4 Gel filtration of 5T4 antigenic molecules. Solubilised StMPM protein fractionated over S200 Sephacryl in the presence of detergent. Fractionated 5T4 antigenicity assessed in ELISA (■——■).

family of mucin type glycoproteins (Swallow *et al.*, 1987), but with clear differences from those defined by the CA or HMFG series of antigens (Wiseman *et al.*, 1984; Burchell *et al.*, 1983). These latter glycoproteins are defined by several monoclonal antibodies which have been shown to be reactive with a wide range of malignant tumour cells but also reactive with certain specialised normal epithelia.

The limited tissue distribution and expression by selected tumour cell lines encourages further studies on the expression of 5T4 antigen by solid tumours of diverse origin. Analysis of primary tumour material from a variety of neoplasms has revealed specific staining of some different tumours (Southall, Boxer, Bagshawe *et al.*, in preparation). The 5T4 antigenicity appears to depend on both protein and carbohydrate structures but studies using a radiobinding assay with a teratocarcinoma cell line suggested that fixation procedures do not destroy 5T4 antigenicity. Thus immunohistological analysis of 5T4 may be possible using fixed and embedded material.

Table IV Effect of enzymic digestion on 5T4 antigenicity assessed in immunodot

Enzyme	*5T4 titre*
PBS	80 ng
Pronase	>10 μg
Trypsin	>10 μg
Neuraminidase	80 ng
N-glycanase	>10 μg

StMPM protein incubated overnight at 37°C with appropriate enzymes or PBS (as control for autodegradation) and dot-blotted onto nitrocellulose. Results expressed as minimum protein dot concentration required to produce a positive result.

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